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Physics in Biology UCRL-PROC-211198

Measurements of Protein Folding Kinetics Enabled by Fast Diffusive Mixing

Olgica Bakajin

Measurements of the kinetics of protein folding on the microsecond time scale are enabled by the development of microfluidic mixers, which allow us to access conformational changes of the protein under conditions far from equilibrium and at previously inaccessible time scales. The fastest mixing time we have reported is 8□m, while reducing the sample consumption to femtomoles. In a single experiment, we can record time scales from under 10□m to over 100□ms.

To achieve mixing on the microsecond time scale, we reduce the length over which the molecules need to diffuse by using hydrodynamic focusing. The equation that describes this diffusion was formulated by Einstein and explains how complex fluids, like the denaturant that prevents the protein from folding, diffuse into water. The equation was derived from the then still controversial idea of atoms and the observation that tiny pollen particles observed through a microscope can be seen to jiggle about (Brownian motion).

This poster describes the design and optimization of the mixers, using modeling of convective diffusion phenomena, and characterization of the mixer performance, using microparticle image velocimetry, dye quenching, and fluorescent resonance energy-transfer measurements of single-stranded DNA.

Physics of Biomineralization

Chris Orme

Biological systems use peptides and proteins to direct the formation of mineralized tissue, thereby creating complex shapes and hierarchical composites not obtainable in the laboratory. Force microscopy is being used to understand the physical controls on biomineralization by directly imaging the growth of inorganic crystal surfaces in the presence of organic, peptide, and protein modifiers.

Big Physics in Small Spaces: Particle-Fluid Coupling in bioMEMS

David Trebotich

In 1827, British botanist Robert Brown noticed that pollen grains “jiggled” in water when observed under a microscope. He was never able to explain what caused this phenomenon. In 1905, unaware of previous observations, Albert Einstein showed that “according to the molecular-kinetic theory of heat, bodies of microscopically visible size suspended in a liquid will perform movements of such a magnitude that they can be easily observed in a microscope, on account of the molecular motions of heat,” and in

doing so deduced the theoretical description of this so-called Brownian motion from statistical mechanics. Although his most famous formula, $E = mc^2$, comes out of his paper on relativity, his derivation of the diffusion relation in these papers has been the most significant for industry.
Today, we use Einstein's theoretical description of Brownian motion and diffusion to describe many phenomena in fluid dynamics. In biological flows, for example, the presence of large molecules in solution, coupled with the very small scales in state-of-the-art biochemical processors and sensors, brings about a new flow regime that is complex and not well understood. The coupling of fluid and particle dynamics is critical to high-fidelity models of these types of flows. At LLNL we have developed advanced numerical algorithms to model polymer-laden fluids in microfluidic devices. Based on a full coupling of particles and fluid we show that concepts derived in Einstein's seminal work—microscopic, Brownian motion and macro-scale, hydrodynamic viscous forces—play a role in manipulation of molecules in a microprocessor.

Measurement of Stoichiometries of Single Biomolecular Complexes using FRET Photon Statistics

Chris Hollars

We are using single-molecule spectroscopy combined with time-correlated single-photon counting (TCSPC) to make stoichiometric measurements of biolomolecular complexes. Using a TCSPC technique called photon antibunching, we determine the composition of the biomolecular complexes through the statistical analysis of the arrival of individual photons. The observation of this behavior is a benchmark for observing fluorescence from single molecules. We will apply this technique to the analysis of model systems followed by the analysis of protein DNA interactions.

Using Force Spectroscopy to Measure the Impact of Multivalent Binding in Drug-Receptor Interactions

Todd Sulchek

Thermal energy causes rapid motions of biological macromolecules and their surrounding solvent. These molecular motions are known as Brownian motion, which Einstein helped explain in his 1905 paper. Brownian motion is responsible for the formation and breaking of all biological macromolecular bonds, which underpin all biological function. We have developed a method to directly measure these processes in a technique called dynamic force spectroscopy. We used the atomic force microscope to measure the binding forces between single molecule mucin1 (Muc1) protein and an antibody screened against Muc1. Muc1 is overexpressed on cell surfaces in a number of human cancers. Our collaborators at the UC Davis Cancer Center use antibodies to Muc1 as the targeting mechanism for delivery of radioimmunotherapeutic drugs, which consist of several such antibodies tethered to a common radioactive payload. Direct determination of binding

affinities for mono- and multivalent configurations of such drugs is critical for their optimization. Such measurements depend on the ability to differentiate between mono- and multivalent bond formation. By measuring the binding strength as the function of the bond loading rate (dynamic force spectra), we determined energy barriers, thermodynamic off-rates, and the distance to the transition state for simultaneous dissociation of one, two, and three protein–protein pairs. This poster shows that although our measured bond strength scales linearly with the number of molecule pairs, the multivalent configuration leads to a precipitous decrease in the thermodynamic off-rates for the complex dissociation.

Understanding Protein–Small-Molecule Interactions using Computational Methods

Felice Lightstone

Molecular recognition is the foundation of the interactions between a protein and small molecules. Using Newtonian and quantum physics, the atoms of the protein and small molecules can be modeled to predict their specific interactions. We have applied these algorithms to study different small molecules that interact and bind to the estrogen receptor, HLADR10, and tetanus toxin. Using computational methods, we are able to predict which class of molecules will bind to the surface of the proteins. With the proteins represented atomistically, molecular docking is used to predict where and how the small molecules bind the proteins. In collaboration with experimentalists, we have used these methods to help interpret experimental results, to design novel therapeutics, and to develop new reagents for biological assays.

The Mechanoelastic Properties of DNA Toroids

Larry Brewer

During spermiogenesis in mammals, histones in developing sperm chromatin are displaced coincident with the binding of the transition proteins, followed by the protamines, which condense and compact the genomic DNA into toroids. The toroids are further organized into a form that is extremely compact and transcriptionally inert, but whose higher order structure remains unknown. In an effort to understand how protamines and other sperm chromatin proteins contribute to this structure, we have constructed a dual optical trap with force-measuring capability to measure the elasticity of an individual DNA molecule as it is exposed to spermatid basic nuclear proteins in a novel microfluidic flow cell. We are interested in answering the following questions: (1) Do disulfide bonds form only between nearest neighbor protamine molecules bound along DNA, or can they form between any protamine molecules in close proximity to

each other? In the latter case, we expect that disulfide bonds would lock adjacent loops of toroidal DNA together, making it impossible to extend the DNA molecule to its full contour length. (2) In the absence of disulfide bond formation, does protamine still stabilize DNA by binding in the major groove? We will see if the binding of protamine prevents the DNA B-to-S form ("overstretching") transition from occurring. The poster reports on the progress made on these experiments and the interpretations of the results, leading to better understanding of the higher order structure of sperm chromatin

Nano-Traincars Moving down DNA tracks: Single-Molecule FRET and Simulations of a DNA Sliding Clamp Moving along DNA

Daniel Barsky

Like "nano-traincars" on tracks, DNA sliding clamp proteins can "ride" more than 10,000 bases along a DNA double helix (> 3 m linear distance). The clamps can also be driven by protein motors and can carry other proteins along. The clamps accomplish this by encircling the DNA, a unique topology in biology. In the absence of other proteins, DNA sliding clamps apparently slide freely along the DNA, yet analysis of the protein structures reveals many positive charges along the inner ring that should create strong salt bridges with the DNA. Why then do the clamps not remain stationary on the DNA? And how can clamps proceed past sizeable distortions in the DNA double helix? By a combined experimental and computational approach, we have investigated the interactions between DNA and a DNA sliding-clamp protein, the beta subunit of pol III. Our molecular dynamics simulations have illuminated the details of the DNA–protein interactions, and we are using single-molecule fluorescent resonant energy-transfer (FRET) measurements to infer the speed and diffusional character of the clamp's translocation.

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